Synopsis of the thesis

"Structural studies on DNA damage inducible protein 1 (Ddi1) of *Leishmania* and the Rotavirus Nonstructural Protein NSP4"

Structural investigations on the Ddi1 (DNA-damage inducible protein 1) of *Leishmania major* and on the rotavirus nonstructural protein NSP4 were carried out. Ddi1 belongs to the ubiquitin receptor family of proteins. One of its domains is similar to the retroviral aspartic proteinases. It has been shown that this domain is the target of HIV-protease inhibitors that were being used in the treatment of AIDS and it was observed that these drugs effectively controlled opportunistic diseases caused by many parasitic protozoa such as *Leishmania* and *Plasmodium* species. The retroviral protease-like domains present in Ddi1 proteins of these organisms were identified as the targets of these drugs. Structural studies on Ddi1 from *L. major* have been carried out, in an attempt to provide a platform for the design of anti-protozoal compounds. Rotavirus NSP4, the first viral enterotoxin to be identified, is a multifunctional glycoprotein that plays critical roles in viral pathogenesis and morphogenesis. As part of an ongoing project on the structural characterization of NSP4, we determined the structure of the diarrhea-inducing region of this protein from the rotavirus strain MF66.

Chapter 1 presents an overview of Ddi1 and NSP4 of the rotavirus with an emphasis on their structural features. The methods employed during the course of the present work are described in Chapter 2.

Structural studies on the retroviral protease-like domain of Ddi1 (Ddi1-RVP) of *L. major* is presented in Chapter 3. Apart from this domain, Ddi1 of *L. major* also has a ubiquitin-associated and ubiquitin-like domains whereas *P. falciparum* has only the ubiquitin-associated domain. Activity of the full length Ddi1 of *L. major* and the retroviral protease domain of *P. falciparum* using an HIV protease substrate was shown to be inhibited by an HIV protease inhibitor, saquinavir. Binding of saquinavir to the proteins was also confirmed by Biolayer Interferometry studies. The crystal structure of the retroviral protease domain of *L. major* Ddi1 has been determined. It forms a homodimeric structure

similar to that of HIV protease and the reported structure of the same domain from *Saccharomyces cerevisiae*. The loops in Ddi1-RVP are similar to the 'flap' regions of the HIV protease which close-in upon substrate/inhibitor binding; they are visible in the electron density maps, unlike the case of the *S. cerevisiae* protein. Though the native form of the domain shows an open dimeric structure, normal mode analysis reveals that it can take up a closed conformation resulting from relative movements of the subunits. The present structure of Ddi1-RVP of *L. major* with the defined 'flap'-like loops will be helpful in the design of effective drugs against protozoal diseases, starting with HIV protease inhibitors as the lead compounds.

Chapter 4 describes the structural investigations carried out on the diarrhea-inducing region of the nonstructural protein NSP4 of the rotavirus strain MF66 which forms an α-helical coiled-coil structure. Crystal structures of a synthetic peptide and of two recombinant proteins spanning this region showed parallel tetrameric organization of this domain with a bound Ca^{2+} ion at the core. Subsequently, we determined the structure of NSP4 from a different strain as a pentamer without the bound Ca^{2+} ion. This new structure provides more insights into understanding some of the functions of NSP4 such as the release of ions into the cytoplasm and binding to the double-layered particle (DLP). We also established conditions responsible for these structural transitions. The crystal structure of the coiled-coil domain of NSP4 presented in this chapter shows an entirely different structure which is an antiparallel tetramer. This explains our failure to determine the structure by the molecular replacement method using known oligomers. The structure was solved by the Sulphur-SAD method using diffraction data collected with Cr Ka radiation. The study reveals that the structural diversity of NSP4 is not limited. We could relate sequence variations and pH conditions to the differences in oligomeric assemblies. Surface properties of the domain suggest that the new form is likely to interact with different sets of proteins compared to those that interact with the parallel tetramers or pentamers. Further investigations are needed to establish this property.